ijcrr
Vol 04 issue 01
Category: Review
Received on:04/10/11
Revised on:13/10/11
Accepted on:20/10/11

PHOTODYNAMIC THERAPY – A NOVEL APPROACH IN POCKET STERILIZATION

Jannani Muthu, Jaideep Mahendra

Department of Periodontics, Meenakshi Ammal Dental College and Hospital, Chennai

E-mail of Corresponding Author: jaideep_m_23@yahoo.co.in

ABSTRACT

Anti microbial photodynamic therapy is one of the most upcoming treatment approaches in field on dentistry. Applications of PDT in dentistry are growing rapidly: the treatment of oral cancer, bacterial and fungal infection therapies, and the photodynamic diagnosis (PDD) of the malignant transformation of oral lesions. Periodontitis as known is polymicrobial disease and this novel therapeutic approach sounds promising in eliminating the microorganisms when used in adjunct to conventional debridement methods. This technique involves application of a photosensitizer and activating it with a light source of specific wavelength. This system in presence of oxygen creates reactive oxygen species that exerts the classic photodynamic reaction. The advantage of this new approach includes rapid bacterial elimination, minimal chance of resistance development and safety of adjacent host tissue and normal microflora. This review discusses about the general principles, mechanism of photodynamic therapy with additional highlights on its application in periodontal diseases.

Keywords: Antimicrobial photodynamic therapy, periodontitis, photosensitizers, lasers.

INTRODUCTION

There have been many changes & developments in dentistry over the past decade than in the previous hundred years combined, and the pace is accelerating!

Oral cavity is an abode of variety of microorganisms and periodontitis is an infectious disease caused by number of these organisms. Current treatment techniques involve either periodic mechanical disruption of oral microbial biofilms or maintaining therapeutic concentrations of antimicrobials in the oral cavity, both of which are fraught with limitations. The development of alternative antibacterial therapeutic strategies therefore becomes important in the evolution of methods to control microbial growth in the oral cavity. Numerous adjunct conventional antimicrobial therapies have been attempted. One disadvantage of these therapies is development of resistant strains. So alternate antimicrobial treatment modalities have been researched & one such promising alternative is antimicrobial photodynamic therapy[1].

The history dates back over 3000 years when Indians used psoralens in the treatment of vitiligo & the Egyptians employed it in the treatment of leucoderma.

Later It was rediscovered by Western civilization at the beginning of the twentieth century. In 1834, Kalbrunner isolated the chemical bergapten from bergamot oil but did not use it in any therapeutic application[2]. In 1900 Prime, a French neurologist, used eosin orally in the treatment of epilepsy. He discovered that this induced dermatitis in sun-exposed areas of skin. This discovery then led to the first medical application of an interaction between a fluorescent compound and light[3]. It was further developed by the Danish physician, Niels Finsen, who at the turn of the last century described the successful treatment of smallpox using red light. He then went on to use ultraviolet light to treat cutaneous tuberculosis and developed the use of carbon arc phototherapy in the treatment of this condition for which he was awarded a Nobel Prize in 1903[3]. History of photodynamic therapy began when Von Tappeiner,who along with dermatologist Jesionek, used a combination of topical eosin and white light to treat skin tumors. They demonstrated the requirement of oxygen in photosensitization reactions and in 1907 they introduced the term "photodynamic action" to describe this phenomenon. The German physician Friedrich Meyer–Betz performed the first study with what was first called photoradiation therapy (PRT) with porphyrins in humans in 1913. Meyer. In 1989, First Paper on photodynamic therapy was presented at the Congress on *photodynamic therapy* of Tumours[4].

This review elucidates the evolution & the current position of photodynamic therapy, its applications in dentistry, especially in periodontal treatments and its likely impact in future.

COMPONENTS OF PHOTODYNAMIC THERAPY

Photodynamic reaction is a physico-chemical reaction that basically involves 2 components: Photo sensitizer and the activating light source.

Photosensitizers

These are natural or synthetic photoactive compounds that have photosensitizing potential. They function by trapping the light in the form of photons and transferring the energy to other molecules resulting in the liberation of short-lived energetic species that interact with biological systems and produce tissue damage[32].

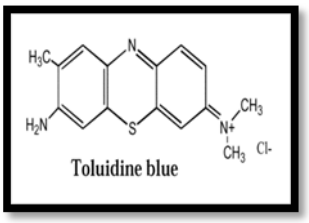
Ideal properties of photosensitizers – this includes photo-physical, chemical, and biological characteristics[5].

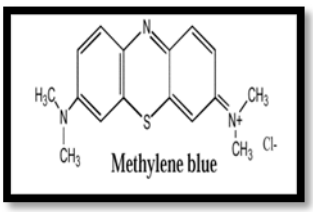
- An ideal photosensitizer must be biologically stable.
- Photochemically efficient.
- Selectively retained in the target tissue.
- Low toxicity and fast elimination from the skin and epithelium.
- Absorption peaks in the low-loss transmission window of biological tissues.
- High quantum yield of singlet oxygen production in vivo.
- Cost-effectiveness and commercial availability
- High solubility in water, injection solutions, and blood substitutes.
- Storage and application light stability.

Generations – Three generations of photosensitizers have evolved over period of time. I generation includes Hematoporphyrins and pthalocyanines, II generation photosensitizers are meso-tetra(hydroxyphenyl)porphyrins marketed as foscan, 5-aminolevulinic acid , tin ethyl etiopurpurin, benzoporphyrin derivative monoacid ring (verteporfin, visudyne) and phenothiazine dyes[5,6,7].

Phenothiazine dyes includes toluidine blue and methylene blue. These are the recent major photosensitizers used in dental field. Both have similar chemical & physiochemical characteristics.

(I).*Toluidine blue* – It's a blue-violet solution. It stains granules within mast cells, proteoglycans & glycosaminoglycans. Used to detect mucosal tumors or atypical epithelia.





III generation dyes are modified by targeting with monoclonal antibodies or with non antibody-based protein carriers and protein/receptor systems, and conjugation with a radioactive tag[7,32]. These include expanded metallo porphyrins, Metallochlorines, Metallopthalocyanines, Metallononpthalocyanines.

Currently, only four photosensitizers are commercially available: Photofrin®, ALA, VisudyneTM (BPD; Verteporfin), and Foscan®. The first three have been approved by the FDA, while all four are in use in Europe.

Light Sources

Numerous light sources have been tried ranging from conventional light lamps, lasers to non laser light sources which have evolved recently. Conventionally lasers

(2).*Methylene blue* – Redox indicator that is blue in oxidizing environments & becomes colorless on reduction. It is used to identify dysplasia or precancerous lesions of oral mucosa. Recently because of its photocatalytic action it is used for virus inactivations before blood transfusions[16]. Both dyes are effective against pathogenic bacteria & are hence used extensively in antimicrobial PDT. They are effective against both gram positive & gram negative periopathogens[8].

have been in use for photoactivation[10,32]. Various lasers that have been used are argon dye lasers, KTP lasers, metal vapor and diode lasers and pumped dye lasers. Most commonly used lasers and wavelengths in intraoral aPDT are Helium-neon lasers – 663 nm, Gallium Aluminum arsenide diode lasers – 630-690 nm, Argon lasers – 488-514 nm. Non laser light sources used are Blue U light systems and light emitting diodes[9, 10].

TECHNIQUES OF ILLUMINATION

3 basic illumination techniques are practiced that includes superficial, interstitial light delivery and intraoperative PDT[3,6].

MECHANISM OF ACTION

The bactericidal effect of photo-dynamic therapy can be explained by two potential, but different, mechanisms. One is DNA

damage and the other is the damage caused to the cytoplasmic membrane of the bacteria by cytotoxic species generated.

The mechanism of action of antimicrobial photodynamic therapy can be briefly described as follows:

After irradiation with light of a specific wavelength (lasers), the photosensitizer at ground state is activated to a highly energized triplet state. The longer lifetime of the triplet state enables the interaction of the excited photosensitizer with the surrounding molecules, and it is generally accepted that the generation of cytotoxic species produced during photodynamic therapy occurs in this state[10,11,12].

The triplet-state photosensitizer follows two different pathways

Type I Reactions

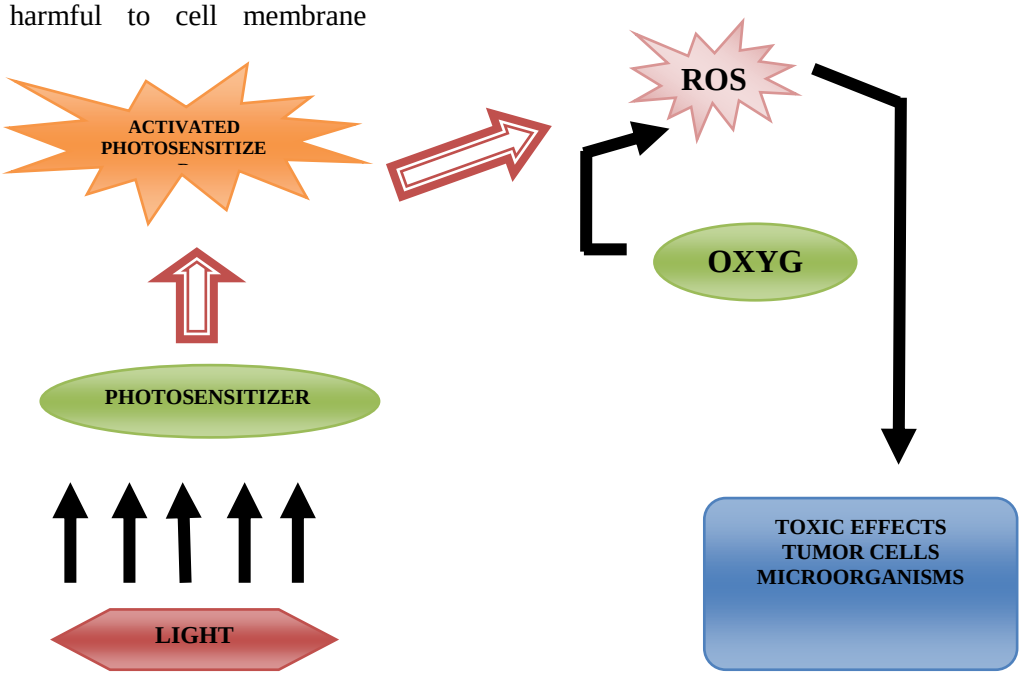
Involve hydrogen-atom abstraction or electron-transfer reactions between the excited state of the photosensitizer and an organic substrate molecule of the cells, which produces free radicals and radical ions. These interact with endogenous molecular oxygen to produce highly reactive oxygen species such as superoxide, hydroxyl radicals and hydrogen peroxide, which are harmful to cell membrane integrity, causing irreparable biological damage[11,12].

Type II Reactions

Involves the production of a highly reactive state of oxygen, known as singlet oxygen ($^1O_2$), which reacts with the surroundings as a result of its high chemical reactivity. The free radicals and the singlet oxygen convey toxic or lethal effects by damaging the cell membrane and the cell wall[10,12].

It seems that the primary cytotoxic agent responsible for the biological effects of the photo-oxidative process is singlet oxygen. Thus, the process of antimicrobial photodynamic therapy is generally mediated by a type II reaction, which is accepted as the major pathway in microbial cell damage[11,12,13].

Microorganisms that are killed by singlet oxygen include viruses, bacteria, protozoa and fungi. Singlet oxygen has a short lifetime in biological systems (<0.04 ls) and a very short radius of action (0.02 lm). The reaction takes place within a limited space, leading to a localized response and making it ideal for application at localized sites without affecting distant molecules, cells or organs[13].



PHOTOINACTIVATION OF BACTERIAL CELLS

Paul Erlikh in the beginning of 20th century came up with the concept of "magic bullet". The bullet is considered as a microbe-targeting drug. It reacts only with a germ, not with the host. He hypothesized that the incubation of bacteria with the methylene-blue dye should cause their death at light exposure[15].

In the 1990s, it was observed that there was a fundamental difference in susceptibility to PDT between Gram-positive and Gram-negative bacteria. The photosensitizer molecules bind in a greater extent to cell wall of gram positive cells whereas it binds to a lesser extent only to outer cell wall layer of gram negative cells. The affinity of negatively charged photosensitizers for Gram negative bacteria may be enhanced by linking the sensitizer to a cationic molecule, by the use of membrane-active agents, or by conjugating the sensitizer with a monoclonal antibody that binds to cell-surface-specific antigens[14,12]. PS does not have to penetrate the bacterium to be effective, or, indeed, even come into contact with the cells. If singlet oxygen can be generated in sufficient quantities near to the bacterial outer membrane, it will be able to diffuse into the cell to inflict damage on vital structures[12,16,17].

PHOTOINACTIVATION OF FUNGI AND VIRUSES

Many studies conducted revealed that lipid-enveloped viruses are more susceptible to PDT than non enveloped strains[10].

Membrane damage and the consequent increased permeability was the proximate cause of cell death after Methylene Blue-mediated PDT on yeast. Human pathogenic parasites have also been killed by combinations of photosensitizers and light[16].

USE OP PHOTODYNAMIC THERAPY IN PERIODONTICS

The main objective of periodontal therapy is to eliminate deposits of bacteria and bacterial niches by removing the supragingival and subgingival biofilm. It has been demonstrated that conventional mechanical therapy cannot completely remove all periodontal pathogens, as the bacteria invade soft tissues also. Systemic use of antibiotics may be recommended in certain situations as an adjunct to periodontal therapy[16]. But the main disadvantage is increased emergence of antibiotic resistant bacterial strains. Photodynamic therapy is considered as one another novel approach to assist mechanical periodontal therapies in tackling the bacterial challenge[10].

The photosensitizer is placed directly in the periodontal and peri-implant pocket and the liquid agent can easily access the whole root or implant surface before activation by the laser light through placement of the optical fiber directly in the pocket[18]. Photodisinfection of periodontal pocket is carried out in following sequence:

1. Initial mechanical debridement using hand curettes.
2. Photosensitizer is applied via syringe at the diseased site that contains residual bacteria. Occasionally, excess dye solution is removed using water spray.
3. Photosensitization is performed using an intensive light by a special tip applied in the pocket. Singlet oxygen and other very reactive agents that are toxic to bacteria are produced, resulting in photochemical disinfection of the periodontal pocket[10,16].

EFFECT OF PDT ON ORAL BIOFILMS

The antimicrobial activity of photosensitizers does not only target bacterial cells but also the oral biofilm

matrix. It has a direct effect on the polysaccharides on the extracellular matrix on biofilms making the bacterial cells more susceptible to photodamage. Such dual activity is not exhibited by antibiotics[17].

PHOTODYNAMIC THERAPY IN TREATMENT OF PERIIMPLANTITIS

The incidence of peri-implantitis in patients with chronic periodontitis is up to five times greater than in patients who are free of this disease[19]. Treatment of peri-implantitis has become an interesting topic among clinicians and researchers. Conventional mechanical methods such as adjunctive application of systemic or local antibiotics and antiseptics has been generally recommended and are apparently ineffective for complete debridement of the bone defect as well as of the contaminated micro structured implant surface[20,21].

Recently lasers and aPDT has gained popularity in treating peri-implant diseases. Numerous studies have shown promising results[19,20,21].

Table: Systematic Review of Photodynamic Therapy

AUTHOR	STUDY	RESULTS
Dobson et al, 1992[22]	In vitro study to determine effects of toludine blue and methylene blue mediated aPDT in biofilms containing P.g, F.n, A.a	Effectively eliminated periodontopathic bacteria from biofilm
Wilson et al, 1995[23]	To determine whether bacteria in supragingival plaque samples could be killed by low-power laser light in the presence of a suitable photosensitizer.	Following irradiation, substantial reductions were achieved in the total anaerobic count as well as in the number of viable streptococci and actinomyces present in the samples
Packer S et al, 1999[24]	To determine the effects of irradiating the organism with red light in the presence of TBO on its proteolytic enzyme activity in suspensions of *P. gingivalis*	On exposure to 126 J of red light in the presence of 12.5 µg/ml of TBO the proteolytic enzyme activity was reduced by 100%.
Dortbudack O et al, 2001[25]	To examine the clinical effectiveness of PDT in reducing periodontal pathogens, such as *A.a, P.g & P.i*	This combined treatment showed significant bacterial reduction, but complete elimination of all three microorganisms was achieved in none of the cases.
Komeric et al, 2003[26]	Inoculated P.g into maxillary molar of rats and did a study to determine whether PDT could be used to kill the organism in the oral cavities of rats and whether this would result in a reduction in the alveolar bone loss characteristic of periodontitis.	When toluidine blue was used together with laser light there was a significant reduction in the number of viable *P. gingivalis.* The bone loss in the animals treated with light and toluidine blue was found to be significantly less.
Oliviera RR, 2007[27]	split mouth design to treat 10 patients with either PDT or SRP. Clinical assessment of PI, GI, PD, BOP, GR & CAL were made at baseline & 3 months after treatment.	Both PDT & SRP showed similar results in non surgical treatment of aggressive periodontitis.
Qin YL, 2008[28]	Conducted a study to investigate the *in vivo* photosensitization of periodontal bacteria in rats and to compare its efficacy with that of routine scaling and root planning	Both PDT & SRP showed similar results

Anderson R 2007[29]	To compare the effectiveness of a photodisinfection process to that of scaling and root planing (SRP) for non-surgical periodontal treatment.	No difference in any of the investigated parameters was observed at baseline between the three groups. At 6 & 12 weeks PDT group showed significant BOP & PPD compared to SRP group. CAL showed no statistical difference between the 2 groups.
Braun et al, 2008[30]	Conducted a study to assess the effect of adjunctive antimicrobial photodynamic therapy in chronic periodontitis. Relative attachment level (RAL), probing depths (PDs) and gingival recession (GR) were evaluated at baseline and 3 months after treatment.	Values for RAL, PD, SFFR and BOP decreased significantly 3 months after treatment in the control, with a higher impact on the sites treated with adjunctive aPDT.
Christodoulides N, , 2008[31]	To evaluate the clinical and microbiologic effects of the adjunctive use of PDT to non-surgical periodontal treatment.	At 3 and 6 months after treatment, there were no statistically significant differences between the groups with regard to CAL, PD, FMPS, or microbiologic changes. At 3 and 6 months, a statistically significantly greater improvement in FMBS was found in the test group.
Oliveria RR, 2009[32]	A split mouth study to investigate cytokine levels in GCF of patients with aggressive periodontitis, after treatment with PDT or SRP. GCF samples were collected & concentrations of TNF α and RANKL were determined by using ELISA.	PDT & SRP had similar effects on TNF α & RANKL in aggressive periodontitis patients
Sigusch BW, 2010[33]	To clinically and microbiologically evaluate the effect of photodynamic therapy (PDT) as a full-mouth procedure in Fusobacterium nucleatum–infected patients with periodontitis.	Four and 12 weeks after PDT, the mean PD and CAL showed significant differences from baseline values and from those of the control group. In the PDT group, 12 weeks after treatment, the F. nucleatum DNA concentration was found to be significantly reduced compared to the baseline level.
Ramanoz et al, 2010[34]	To examine the effects of PDT on the periodontal bacteria in combination with scaling and root planing (SRP) in the same group of patients by randomly selecting PDT or SRP for use in different quadrants of the mouth. For the present study, PDT was compared with a diode laser (980 nm) and an Nd:YA G laser (1,064 nm). Microbiological samples were examined and evaluated over a period of three months	Significant bacterial reduction has been observed in all cases. The diode laser with SRP presented long-term positive results, while PDT showed a significant bacteria reduction during the entire observation period.
Liu J et al, 2011[35]	Split mouth Short-term clinical trial to evaluate the effects of a combination of photodynamic therapy with low-level laser therapy as an adjunct to nonsurgical treatment of chronic periodontitis. PI, BOP, PD & gingival recession were recorded at baseline, 1 and 3 months after the treatment. Gingival crevicular fluid was collected for assay of interleukin-1β levels at baseline, 1 wk and 1 month.	A significant decrease in gingival crevicular fluid volume was observed in both groups at 1 week, with a further decrease at 1 mo in the test sites. The test sites showed a greater reduction of interleukin-1β levels in gingival crevicular fluid at 1 wk than the control sites. No significant differences in periodontal parameters were found between the test and control teeth at 3 months.

REFERENCES

1. Raghavendra M, Bhola S, Koregol A. Photodynamic therapy: A target therapy in periodontics. *Australian dental journal*, 2009; 54(1); 102 – 109.
2. Epstein, J. M. (1990) *Phototherapy and photochemotherapy*, 1990; N. Engl. J. Med. 32, 1149-1151.

3. Rajvir M & Anish; PDT – A strategic review. *Indian journal of dental research*, 2010; 21 (2); 285 – 291.
4. Ackroyd R, Kelty C, Brown N. The History of Photodetection and Photodynamic Therapy, *Journal of Photochemistry and Photobiology* 2006; 74 (5); 656–669.
5. Bown, SG. Tralau, Smith C , Akdemir D, Wieman TJ. Photodynamic therapy with porphyrin and phthalocyanine sensitisation: quantitative studies in normal rat liver. *Br. J. Cancer* 1986; 54, 43-52.
6. Zheng Huang, M.D., Ph.D. A Review of Progress in Clinical Photodynamic Therapy. Technol Cancer Res Treat. 2005 June; 4(3): 283–293.
7. Solban N, Rizvi I, Hasan T. Targeted photodynamic therapy. *Lasers Surg Med* 2006; 38:522-531.
8. Komerik, Goslinski T, in vivo killing of p.gingivalis by toluidine blue mediated photosensitization, *Antimicrobes agents chemother* 2003 : 47:932-940.
9. Whitehurst C, Byrne B, Moore JV. Development of an alternative light source to lasers for photodynamic therapy - Comparative in vitro dose response characteristics. *Lasers Med. Sci.* 1993; 8, 259-267.
10. Takasaki AA, Aoki A, Mizutani K, Schwarz F, Sculean A. Application of antimicrobial photodynamic therapy in periodontal and peri-implant diseases. *Perio 2000*, vol-51, 109-140.
11. Sharman WM, Allen CM, Van Lier JE. Role of activated oxygen species in photodynamic therapy. Methods in Enzymology 2000; 319; 376-400.
12. Nikolaos s. Soukos j & Max goodson. photodynamic therapy in the control of oral biofilms. P*eriodontology 2000*, vol. 55, 2011, 143–166 .
13. Busch TM, Local physiological changes during photodynamic therapy. *Lasers Surg Med*,2006, 38:494-399.
14. Hamblin MR and Hasan T. Photodynamic therapy: a new antimicrobial approach to infectious disease. *Photochem . photobiol . Sci*, 2004; 3, 436 – 450.
15. Wainwright M.J. *Antimicrob. Chemother*.1998; 42; 13-28.
16. Konopa K & Golinsky T; Photodynamic therapy in dentistry. *J Dent Res*, 2007; 86 (8); 694 – 704.
17. Maisch T, Bosl C, Szeimies RM. Photodynamic effects of novel XF porphyrin derivatives on prokaryotic and eukaryotic cells. *Antimicrob Agents Chemother* 2005;49(4):1542–52.
18. Herbert L. Vascular Targeted Photodynamic Therapy for Localized Prostate Cancer, *Rev Urol*. 2008 Fall; 10(4): 254–261.
19. Haas R, Dörtbudak O, Mensdorff NP, Mailath G. Elimination of bacteria on different implant surfaces through photosensitization and soft laser - An in vitro study. *Clinical Oral Implants Research* 1997; 8 (4); 249 – 254.
20. Jamil AS, Martins MC, Nociti FH jr, Garcia VG. Treatment of ligature-induced peri-implantitis by lethal photosensitization and guided bone regeneration: a preliminary histologic study in dogs. *J Periodontol* 2003; 74(3); 338 – 345
21. Hayek RR, Araújo NS, Gioso MA, Ferreira J, Baptista-Sobrinho CA, Yamada AM, Ribeiro MS. Comparative study between the effects of photodynamic therapy and conventional therapy on microbial reduction in ligature-induced peri-implantitis in dogs. *J Periodontol* 2005; 76(5), 1275 – 1281.

22. Dobson J & Wilson M. Sensitization of oral bacteria in biofilms to killing by light from a low-power laser. *Arch oral biol*; 1992; 32; 883-887.
23. Wilson M, Burns T, Pratten J, Pearson GJ. Bacteria in supragingival plaque samples can be killed by low-power laser light in the presence of a photosensitizer. *J Applied Bacteriol.* 1995; 17(5); 569 – 574
24. Packer S, Batthi M, Burns T, Wilson M. Inactivation of Proteolytic Enzymes from Porphyromonas gingivalis Using Light-activated Agents. *Lasers Med Sci* 1999; 15(1); 24 – 30
25. Dörtbudak O, Haas R, Bernhart T, Matejka M. Photodynamic Therapy for Bacterial Reduction of Periodontal Microorganisms 2001; 1(2); 115 – 118.
26. Kömerik N, Nakanishi H, MacRobert AJ, B. Henderson. In Vivo Killing of *Porphyromonas gingivalis* by Toluidine Blue-Mediated Photosensitization in an Animal Model. *Antimicrob Agents Chemother*. 2003; 47(3): 932–940
27. Oliveira RR, Schwartz HO, Novaes BA Jr, and Taba M Jr. Antimicrobial Photodynamic Therapy in the Non-Surgical Treatment of Aggressive Periodontitis: A Preliminary Randomized Controlled Clinical Study. *J Periodontol 2007*; 78(6), 965 – 973.
28. Qin YL, Luan XL, Sheng YO. Comparison of toluidine blue-mediated photodynamic therapy and conventional scaling treatment for periodontitis in rats. *J periodontal research* 2008; 43(2); 162 – 167.
29. Roger Andersen, MD, MPH Nicolas Loebel, PhD David Hammond, BS. Treatment of Periodontal Disease by Photodisinfection Compared to Scaling and Root Planing. *J Clin Dent* 2007; 18(2); 1 – 5.
30. Braun A, Dehn C, Krause F, Jepsen S. Short-term clinical effects of adjunctive antimicrobial photodynamic therapy in periodontal treatment: a randomized clinical trial. J Clin Periodontol 2008; 35(10); 877 – 874.
31. Christodoulides N, Nikolidakis D, Chondros P, Becker J, Schwarz F, Rössler R, Sculean A. Photodynamic therapy as an adjunct to non-surgical periodontal treatment: a randomized, controlled clinical trial. J Periodontol 2008; 79(9); 1638 – 1644.
32. Oliveira RR, Oswaldo HS, Arthur BN Jr, Garlet GP. Antimicrobial Photodynamic Therapy in the Non-Surgical Treatment of Aggressive Periodontitis: Cytokine Profile in Gingival Crevicular Fluid, Preliminary Results. Journal of Periodontology 2009, 80(1); 98-105.
33. Sigusch BW, Engelbrecht M, Völpel A, Holletschke A, Pfister W, Schütze J. Full-mouth antimicrobial photodynamic therapy in Fusobacterium nucleatum-infected periodontitis patients. J Periodontol. 2010;81(7):975-81.
34. Romanos GE, Brink B. Photodynamic therapy in periodontal therapy: microbiological observations from a private practice. Gen Dent. 2010 ;58(2):68-73.
35. Lui J, Corbet EF, Jin L. Combined photodynamic and low-level laser therapies as an adjunct to nonsurgical treatment of chronic periodontitis. J Periodont Res 2011; 46: 89–96.
36. Allison NY. Photosensitizers in clinical PDT. *Photodiagn Photodyn Ther* 2004; 7; 27-42.
37. Augthun M, Tinschert J, Huber A. In vitro studies on the effect of cleaning methods on different implant surfaces. *J Periodontol* 1998: 69: 857–864.